PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	) :	Examiner: Christine E. Foster
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Application No.: 10/539,797	) :	
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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

THIRD PARTY SUBMISSION OF ART UNDER 37 C.F.R. § 1.99

Sir:

In accordance with the practice under 37 C.F.R. § 1.99, it is respectfully requested that the following publications be entered in the application file and considered by the Examiner. The fee set forth in 37 C.F.R. § 1.17(p), a copy of each of the listed documents, and a certification that the information being submitted has been served upon the applicant in compliance with 37 C.F.R. § 1.248(b) are included herewith. The Office is respectfully requested to return the enclosed self-addressed postcard to acknowledge that this submission has been received (37 C.F.R. § 1.99(f)).

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REFERENCES

Reference 1: K. Arvesen et al., "Calprotectin: a novel plasma marker of granulocyte activation in acute coronary syndrome", Abstract Book of the XVIII Congress of the European Society of Cardiology, August 25-29, 1996, p. 429, No. 2335.

Reference 2: B. Johne et al., "Functional and clinical aspects of the myelomonocyte protein calprotectin", Molecular Pathology, June 1997, Vol 50, No. 3, p. 113-123.

Reference 3: G.J. Blake and P.M. Rider, "Novel Clinical Markers of Vascular Wall Inflammation", Circulation Research, October 26, 2001, p. 763-771.

It is respectfully requested that the references enclosed with this submission be entered in the application file and considered by the Examiner with regards to the above-referenced patent application. It is further requested that the self-addressed postcard be returned, indicating that this submission has been received.

The undersigned attorney may be reached in our Washington D.C. office by telephone at (202) 530-1010. Any correspondence should be directed to the address given below.

Respectfully submitted,

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CERTIFICATE OF SERVICE

I hereby certify that the foregoing THIRD PARTY SUBMISSION OF ART UNDER 37 C.F.R. § 1.99 is being served on the below-named attorney for applicant this 14th day of August, 2006, via first class mail, postage prepaid, addressed to:

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Jeffrey M. Connor

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XVIIIth Congress of the European Society of Cardiology, Birmingham, United Kingdom, August 25-29, 1996. Abstract book.

2332 Lack of the reverse use-dependent 'effect of the class-III antiarrhythmic drug' d-sotalol on the guinea pig epicardium

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ost of the experimental and clinical studies on endocardial tissue with d-talol have shown a decreasing effect on the action potential duration with creasing stimulation rates (reverse "use-dependency"). We studied the effect d-sotalol (10^{-5}, 3×10^{-5}, 10^{-4}, 2×10^{-4} M) on intracellular action potentials the epicardium of the guinea pig at an extracellular potassium concentration 3.5 M.

Results: The prolongation of the action potentials (ΔAPD) at 90% repolariza-n at different stimulations rates are as follows (n = 6, Means ± SD, * = p < 05 vs. 0.1 Hz):

APD90	10^{-5}	3×10^{-5}	10^{-4}	2×10^{-4}
1 Hz	10 ± 7 msec	17 ± 9 msec	17 ± 10 msec	15 ± 8 msec
3 Hz	12 ± 9 msec	19 ± 10 msec	19 ± 12 msec	21 ± 8 msec
Hz	13 ± 8 msec	31 ± 16 msec	31 ± 14 msec	35 ± 11 msec*
Hz	3 ± 2 msec	13 ± 6 msec	36 ± 12 msec*	39 ± 15 msec*

Conclusion: 1.) Compared to the reverse "use-dependent" effect of d-sotalol in ndocardium, in epicardium the drug prolongs the action potential duration with ositively "use-dependent" pattern. 2.) Due to differential effects endocardially d epicardially d-sotalol enhances the dispersion of the refractory periods. 3.) is implies a possible proarrhythmic potential of d-sotalol.

2333 The 'reverse-use dependence' of the class III antiarrhythmics can be prevented by decreasing intra-cellular calcium

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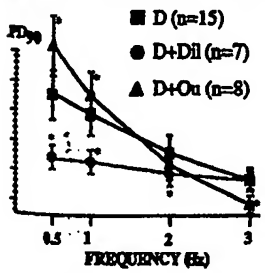
everse-use dependence" makes class III antiarrhythmics more prone for arrhythmias and less likely to control successfully the tachyarrhythmias. This enomenon is related, at least in part, to the summation of nondeactivated I_{Ks} rapid heart rates. The magnitude of this current depends on the intracellular $^{2+}$ ($[Ca_i^{2+}]$). We tested the hypothesis that changing $[Ca_i^{2+}]$ could modify this enomenon.

Transmembrane action potentials (AP) were recorded by means of standard croelectrode techniques in guinea-pig right ventricular papillary muscle. AP ration at 90% repolarization (APD_{90}) were evaluated at 0.5, 1, 2 and 3 Hz, control and 30 min after dofetilide (D) 10 nM. Then, either DH Ouabain (Ou) μM, or diltiazem (Dil) 10 μM was added into the bath solution, in order to crease, or decrease $[Ca_i^{2+}]$, and measurements were repeated after 30 min. e prolongation (means ± SEM, ms) of APD_{90} (ΔAPD90) by D, D + Dil and + Ou, at each pacing frequency, is shown in the following graphic: * $p < 0.01$ mpared to D. With increasing $[Ca_i^{2+}]$ (Ou), the more pronounced effect of D 0.5 Hz was almost completely abolished at 3 Hz. In decreased $[Ca_i^{2+}]$ (Dil), effect of D is reduced at slow, but not affected at rapid pacing rates.



So, decreasing $[Ca_i^{2+}]$ prevents "reverse-use dependence" of D, making its tiarrhythmic profile clinically more favourable.

LEUCOCYTE ACTIVATION IN ACUTE CORONARY SYNDROMES

2334 Neutrophil activation in unstable angina is not related to activation of the haemostatic system

C. Monaco, G. D'Onofrio [1], G. Liuzzo, G. Caligiuri, G. Zini [1], A.G. Rebuzzi, G. Quaranta, A. Meo, L.M. Biasucci, A. Maseri. *Institute of Cardiology;* [1] *Institute of Haematology, Catholic University, Rome, Italy*

To assess whether neutrophil degranulation in unstable angina (UA), is caused by activation of the thrombotic system, we studied the time course of the intracellular index of myeloperoxidase (MPXI), a marker of neutrophil activation, in relation to levels of Thrombin-anti thrombin III complexes (TAT) and of Fragment 1 + 2 (F1 + 2) as markers of activation of the coagulation system in 20 patients (pts) with UA and in 20 healthy volunteers (N). Samples were taken on admission, and at 6, 24, 48 and 72 hours. In all samples levels of C-Reactive Protein (CRP) were also measured.

Results (median and range): On admission CRP and MPXI levels were significantly different in the 2 groups (UA vs N): CRP 4.6 mg/l (range 0.4/–82) vs 1.8 mg/l (range 0.7/5.7, $p < 0.01$), MPXI –4 (range +5/–15) vs –1.3 (range +5.5/–7, $p < 0.01$); no differences were found between UA and N in TAT e F1 + 2 values: TAT: 2.05 ng/ml (range 0.5/–14.4) vs 2.1 ng/ml (range 1.05/4.2); F1 + 2: 0.83 nmol/l (range 0.14/–1.65) vs 0.59 nmol/l (range 0.41/0.98). During the study 11 episodes of elevation of TAT and/or F1 + 2 (6 symptomatic and 5 asymptomatic) followed by at least one sample for MPXI were observed. No changes in MPXI values after the episodes of activation of the coagulation as compared to baseline values were observed (–3.5, range –11.4/1.2 vs –4.7, range –13.1/4.5 respectively). There was no correlation between MPXI and TAT, and MPXI and F1 + 2, while a significant correlation was found between MPXI and CRP ($r = -0.5$, $p = 0.03$).

Conclusions: Our data suggest that systemic neutrophil activation in unstable angina is not related to activation of the coagulation system and may be related to a primary cause of instability.

2335 Calprotectin: a novel plasma marker of granulocyte activation in acute coronary syndrome

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The inflammatory activity associated with atherosclerotic plaque rupture includes activation of polymorphonuclear leukocytes (PMNs). The aim was to study plasma calprotectin, a calciumbinding protein released by activated PMNs, in patients (pts.) with unstable angina pectoris (UAP) or non-Q myocardial infarction (NQMI).

Methods. Calprotectin was measured in 286 pts. with either UAP or NQMI randomised in a placebo-controlled, double-blind trial of low molecular weight heparin (Fragmine®). Calprotectin was measured on admission (t1) and after 48 hrs. (t2). The reference material consisted of 204 healthy volunteers.

Results. Plasma Calprotectin (mg/L)

Results. Plasma calprotectin (mg/L)

	n	Mean	SD	P value
Reference	204	0.62	0.19	< 0.0001
all pts. at t1	286	2.60	2.22	
UAP, t1	165	2.56	1.98	0.032
NQMI, t1	121	3.11	2.49	
UAP, t2	141	2.44	1.78	0.001
NQMI, t2	106	3.53	2.95	

Conclusion. Pts. with acute coronary syndrome have elevated calprotectin levels indicating activation of PMNs. These findings support that inflammatory mechanisms are of importance in unstable angina and acute myocardial infarction.

Reviews

Functional and clinical aspects of the myelomonocyte protein calprotectin

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Calprotectin is a calcium binding heterocomplex protein consisting of two heavy and one light chain.[1 2] It belongs to the S-100 protein family and is derived predominantly from neutrophils and monocytes.[1] This protein is distributed in myelomonocytic cells, epithelial cells, and keratinocytes and in various tissues and fluids in the body,[1] and is a putative protective protein.[3 4] Calprotectin and its subunits appear to have regulatory functions in the inflammatory process,[5 6] and various biological functions including antimicrobial[7–9] and antiproliferative[10 11] activity have been ascribed to the protein. In acute phase inflammatory reactions calprotectin is detectable in elevated amounts that, in some instances correlate to elevated levels of neutrophil granulocytes or other inflammation parameters such as C reactive protein (CRP) or erythrocyte sedimentation rate (ESR).[12–14] Sander *et al*[15] found poor correlation between calprotectin and CRP, blood leucocytes, and ESR in life threatening infections, thus suggesting that these parameters reflect separate aspects of the inflammatory response.

The clinical relevance of calprotectin measurements has been described in several disease conditions, particularly in inflammatory diseases and certain microbial infections, as well as neoplastic conditions.[16 17] The highest rise in calprotectin concentrations can be found in cystic fibrosis, rheumatoid arthritis, Crohn's disease, ulcerative colitis, and bacterial infections.[12–14 18 19]

Why do we find calprotectin so fascinating? There are already numerous markers of inflammatory reactions[9 20–26] including granulocyte markers, so why introduce another? Calprotectin is a multipotent biologically active molecule.[1 5] We know that large molecules can have separate domains with different biological functions[27 28] and that cellular functions (in macrophages) can be selectively and simultaneously up or downregulated via different entities on such macromolecules.[28] Calcium binding proteins of the S-100 protein family are involved in complex intracellular signal tranductions,[27 29] and it has been suggested that calprotectin plays an important role in the metabolism of myeloid cells.[30] Furthermore, when calprotectin is external to cells it has immunomodulatory functions[5 22] and an important role in neutrophil defence against microbial infections.[21 31 32] Antitumour activity has also been suggested,[10 11] as well as an innate defence function.[3 4] Thus, calprotectin appears to be an important regulatory protein inside the myeloid cells and extracellularly in inflammatory reactions. This remarkable spectrum of functions demonstrated in a recently discovered protein prompted an up to date review as a basis for further studies.

Table 1 Calprotectin nomenclature

Nomenclature	*References**
Calprotectin (L1) (2 heavy + 1 light chain)	Fagerhol,[1 2] Fagerhol *et al*[33]
MRP 8/14 (MIF related protein)	Odink *et al*[34] Sorg[5]
Cystic fibrosis associated antigen (CFA) (identity with calprotectin and MRP-8)	Wilson,[35 36] Dorin[37] (Anderson *et al*[38 39])
Calgranulins A and B	Wilkinson *et al*[40]
S-100a and b (calcium binding proteins)	Dorin *et al*,[37 41] Freemont *et al*[27]

*First publications and/or key references.

Nomenclature

Calprotectin is an elusive protein with many names[2]; several independent research groups have called it L1 protein, MRP-8/14, calgranulin, and cystic fibrosis antigen (table 1).

Calprotectin was first isolated from granulocytes as described by Fagerhol *et al* in 1980[33] and named L1 protein. The name calprotectin was proposed later[8] when calcium binding and antimicrobial activity had been documented. Sorg's group[5] worked with macrophages and mechanisms of chronic inflammation, and the MIF related proteins MRP-8, MRP-14, and their heterocomplex MRP-8/14 were described in 1987 by Odink.[34] A cystic fibrosis associated antigen was first described by Wilson *et al* in 1973[35] and further characterised by Wilkinson *et al*[40] who proposed the name calgranulin in 1988. The identity between the L1 protein, cystic fibrosis antigen, and MRP-8 and MRP-14 was established in 1988 by amino acid and cDNA sequencing, and immunohistochemical staining.[38 39] Dorin *et al*[37 41] and Freemont *et al*[27] further characterised the S-100 structure of these proteins.

Calprotectin is a suitable descriptive name for this multipotent calcium binding protein with protective properties.[2]

Table 2 Physicochemical properties

Molecular weight 36 kDa[2]
Two heavy chains of 14 kDa[2]
One light chain of 8 kDa[2]
Each chain binds two calcium ions[42]
Zinc binding[43]
Heat resistant[42]
Resistant to proteolysis when calcium is present[1,2]
Immunogenic[44]
MoAb defined epitopes
Mac387[45-47]
27E10[5]
S 36.48[48]
S 32.2, 8-5C2[49]
CF145, CF557[46]
F11, F3, A1[50]
CP-1, -2, -5[51]
Strong bond between subunits[1,2]
Forms noncovalent complexes (-di, -tri, and tetramers) in a calcium dependent manner[2,3,52]
S-100 protein structure and sequence identity[1,17,37,53-55]
Mapped to human chromosome 1 (and murine 3)[41,48,56]

The references cited are representative reports or reviews, not a complete list of publications on each topic.
MoAb, monoclonal antibody.

Table 3 Distribution in cells and tissues

Cells
Neutrophil granulocytes[33,57,58]
Monocytes/macrophages[9,34,54]
Epithelial cells[20,60-62]
Keratinocytes[63-65]
Pancreatic cell lines[66]
Tracheal gland cells[67]
Tissues
Skin (epidermis/dermis)[62,68]
Lung[16]
Gut[69]
Oral mucosa[63,64]
Cervix mucosa[63]
Body fluids[1] (see table 6)
Other species
Mouse[70]
Rat[10,71]
Pig[72]
Sheep[73]
Rabbit[74]

The references cited are representative reports or reviews, not a complete list of publications on each topic.

Structure and physicochemical properties

The physicochemical properties of calprotectin (table 2) have been reviewed thoroughly.[1] It is a 36 kDa heterotrimeric calcium binding protein with two heavy and one light chain noncovalently linked.[1,2,42] In the presence of EDTA it is anionic and migrates in electrophoresis as an α_2 globulin, whereas in the presence of calcium it is slightly basic and migrates as a γ globulin. Free heavy and light chains have been detected only after dissociating treatment, typically by heating in the presence of sodium dodecyl sulphate (SDS) and 8 M urea followed by SDS polyacrylamide gel electrophoresis or two dimensional electrophoresis (isodalt). The polypeptide chains may be purified by isoelectric focusing in the presence of 6 M urea.[57,58] Reactive sulphydryl groups are exposed after dissociation, and unless these are blocked or alkylated, various heterodimers may be formed.[52,59] Addition of calcium may promote formation of such complexes and even calprotectin dimers—that is, double heterotrimers.[57,42]

Epitope mapping of the protein, by use of a series of overlapping seven amino acid long synthetic peptides covering the heavy and light chains, has shown that rabbits produce antibodies against three different linear epitopes on each chain (Hansen *et al*, 1995, unpublished). Many monoclonal antibodies against calprotectin have been produced (table 2). Epitope mapping of calprotectin with six murine monoclonal antibodies revealed four separate epitopes (Johne and Hansen, 1995, unpublished). Binding of calcium causes conformational changes in calprotectin as demonstrated by circular dichroism[42] and reactivity with monoclonal antibodies.[51] Furthermore, calcium makes the protein remarkably resistant against heat and proteolysis.[2,42] Its S-100 like protein structure has been established[1] and the genes have been sequenced and mapped to human chromosome 1, q12–q21.[41,48]

Distribution in cells and tissues

Calprotectin is found in cells, tissues, and fluids in all parts of the human body (table 3). It is mainly a myelomonocyte and keratinocyte protein. In neutrophils, calprotectin is located in the extralysosomal cytosol in concentrations estimated at 5–15 mg/ml,[33,57] thus constituting about 5% of total proteins in neutrophil granulocytes. By immunoelectron microscopy, small amounts were also found in electron dense parts of myelomonocyte nuclei (Stäubli and Fagerhol, 1991, unpublished). The calprotectin chains are expressed in monocytes and activated macrophages but in decreasing amounts with increasing differentiation of the latter. Calprotectin is variably expressed on the surface membrane of granulocytes and monocytes.[75] Dendritic cells may be induced to L1 expression in certain reactive states.[1] Various mucosal squamous epithelia have been shown to express cytoplasmic calprotectin even in the normal state.[60] Merten and Figarella[67] reported calprotectin secretion in tracheal gland cells, and Fanjul *et al*[66] demonstrated MRP-8 and MRP-14 in pancreatic cells. Calprotectin is also found in rat, mouse, rabbit, sheep, cattle, and pig.[10,70-74,76] It is thus an abundant ubiquitous molecule.

Biological function

Recent publications have revealed numerous biological functions of the calprotectin molecule in vitro and in vivo (table 4). The structural identification as an S-100-like protein and its calcium dependent association to cytoskeleton structures[29] suggests intracellular signal transduction functions.[87] Translocation has been shown of phosphorylated calprotectin chains to the membrane during human neutrophil activation,[88] and inhibition of intracellular enzymes important in cell proliferation (casein kinase II and topoisomerases).[89] It was demonstrated that the protein is phosphorylated, and this raises the possibility that calprotectin may be a competing substrate for the enzyme. The first documentation of antimicrobial activity[8] has been confirmed in several papers (table 4), and has given calprotectin a central role in neutrophil defence.[21,90,91] The abundance and distinctive properties of calprotectin suggests that it plays an important role in neutrophil biology.[32,92] Stimulation of immunoglobulin production,[6] chemotactic factor activity,[83] and

Table 4 Biological functions

Intracellular signal transduction (S-100 function)[27 47 77]
Calcium dependent association to cytoskeleton structures[29 77]
Antimicrobial activity[8 21 31 78 79]
Anti *Candida albicans* activity[33 80-82]
Neutrophil defence mechanism[21 32]
Stimulation of immunoglobulin production[5]
Chemotactic factor[83]
Neutrophil immobilising factor[27]
Regulatory protein in inflammatory reactions[5]
Marker of myelomonocytic cell differentiation[84 85]
Cytotoxic effects:
a) cytotoxic factor in rat peritoneal excudate cells[10]
b) induction of apoptosis[11]
Protective action against rheumatoid arthritis (rat model)[86]

The references cited are representative reports or reviews, not a complete list of publications on each topic.

neutrophil immobilising factor activity[27] are also functions in a non-specific defence repertoire, and related to its properties as a regulatory protein in inflammatory reactions.[13] Calprotectin is a marker for myelomonocytic cell differentiation,[84 85] and it has cytokine-like effects.[10 11] Brun *et al*[86] demonstrated protective action of injected calprotectin in a rat model of avridin induced rheumatoid arthritis, confirming its regulatory role in inflammatory reactions. Sorg[5] discussed the importance of the single protein chains versus the heterocomplex in chronic inflammation.

Data by Hahn and Sohnle,[9] Clohessy and Golden,[81 82] and Sohnle *et al*[93] suggest that the antimicrobial action is at least in part the result of binding to zinc. It has been shown[89] that calprotectin may inhibit metalloproteinases, which may also involve deprivation of zinc. The zinc binding site is separate from the calcium binding sites on MRP-14.[43] Thus calprotectin is a large multipotent biologically active molecule with defined regions or epitopes with distinct functions. There is, however, much to be done regarding the investigation of structure effect relations. Particularly exciting are the conformational changes related to calcium binding, and its importance for molecular functions.

Clinical relevance

The mere fact that no calprotectin deficiency has been found (among more than 5000 individuals tested) may suggest that this protein is of vital importance. The clinical relevance of calprotectin in a number of disease conditions has been suggested by several authors (table 5). The first measurements of concentration[33] in plasma from normal subjects and from patients with inflammatory, infectious, and malignant diseases have been followed by extensive documentation with immunohistochemical methods, and with immunometric concentration measurements in various body fluids (table 6). In several disorders the differences between normal and pathological levels are large enough to suggest a diagnostic potential for calprotectin.[15 17 18 97 105 106 118]

Early attention was paid to rheumatic diseases.[13 35 94] Cystic fibrosis was studied before the protein was identified and characterised, and several other inflammatory diseases have since been studied (table 5). The relevance of calprotectin in malignant diseases has also been pointed out.[16 17 50 123]

CALPROTECTIN IN PLASMA

Increased concentration of calprotectin in plasma is found in many types of infectious or organic diseases.[15] This seems logical in view of the large amounts of the protein in myelomonocytic cells, their active role in defence against infections, and their participation in inflammatory processes and removal of dead cells. We therefore hypothesised that plasma calprotectin concentrations reflect the turnover of myelomonocytic cells in the body. Plasma concentrations during viral infections rarely exceed 2 mg/l, while in bacterial infections values above 3 mg/l are seen regularly.

In a series of patients with meningococcal infections, calprotectin concentrations at hospital admission were between 10 and 120 mg/l in those with fulminant septicaemia (table 7). As calprotectin is preformed and ready to be released from activated circulating leucocytes, its concentration in plasma can increase much more rapidly in response to bacteraemia and endotoxaemia than acute phase proteins (CRP) synthesised in the liver. The latter has a doubling time of three to four hours, so it may take from six hours to more than a day for the plasma concentration to reach more than the upper reference limit. If a rapid test is developed for use in primary health care, plasma calprotectin determinations may contribute to the distinction between viral and bacterial infections and proper use of antibiotics. Sander *et al*[15] concluded that "low or normal L1 levels argue strongly against bacterial infection, while elevated L1 levels discriminate poorly between bacterial and non-infectious inflammatory or malignant disease."

Upregulation of S-100 protein CP-10 by endotoxin via distinct pathways has been demonstrated in murine cells in vitro.[126] It may be calculated that if most of the calprotectin in neutrophil granulocytes (about 5 pg/cell) in the blood of a healthy human individual (containing about 4×10^9 neutrophils per liter) is released in to the plasma, the concentration is expected to increase from 0.5 to about 20 mg/l.

Table 5 Clinical relevance of calprotectin reported in various disease conditions

Blood donors (plasma reference levels)[113]
Rheumatoid arthritis[12 13 22 94]
Sjøgrens syndrome[91]
Intraocular inflammatory conditions[95]
Systemic lupus erythematosus[96]
Cystic fibrosis[40 97]
Acute and chronic lung disease[98]
Lung carcinoma (squamous cells)[14]
Soft tissue tumor marker (non-specificity)[99 100]
Colorectal cancer[17 101 102]
Crohn's disease[18 103 104]
Ulcerative colitis[19 105 106]
Gastrointestinal mucosal inflammation[107 108]
Urinary stone[109]
Oral inflammatory mucosal disease[64]
CNS inflammatory disease (multiple sclerosis and acute encephalitis)[110]
Secondary CNS infections in HIV infected patients[18]
HIV infected patients[7 111]
Haematological patients[50]
Febrile conditions infectious and non-infectious[15]
Acute myocardial infarction[112]
Surgery[114-116]
Apheresis[117]

The references cited are representative reports or reviews, not a complete list of publications on each topic.

Table 6 Calprotectin concentrations in various body fluids

Body fluid	*References*	*Calprotectin concentrations**
Serum/plasma	Sander *et al*[13]	Infectious diseases Normal 0.1–0.6 Viral 0.1–1.4 Bacterial 0.6–11.0
	Müller *et al*[119]	HIV infection (25-75 percentiles) 1.2–9.4
	Dale[115]	Reference intervals Females 0.09–0.53 Males 0.12–0.66
	Berntzen *et al*[12-14]	Rheumatic disease Normal range 0.8–0.91 Rheumatoid arthritis 1–46 Osteoarthritis 0.5–0.8 Juvenile rheumatoid arthritis 2–24
	Haga *et al*[96]	Systemic lupus erythematosus, mean 3.6
	Semb *et al*[114]	Cardiopulmonary bypass operations Preoperative 0.3 Postoperative 5.2±1.3
	Garred *et al*[115]	Major surgery Preoperative baseline 0.5–0.9 Postoperative (4h) 7–15
	Lügering [103 106]	Crohn's disease Active 17±6 (MRP 8/14) Inactive 5±2 (MRP 8/14)
	Golden *et al*[97]	Cystic fibrosis children Controls 0.3–1.6 (median 0.7) Cystic fibrosis 0.4–26 (median 1.8)
	Ivanov *et al*[50]	Haematology Healthy blood donors 0.2 Leukaemia 0.4–13.3 (MRP 8/14)
Cerebrospinal fluid	Dunlop *et al*[18]	Normal range 0–37 µg/l HIV positive with infections 30–350 µg/l
Oral fluids	Brun *et al*[91]	Sjøgren's syndrome Stimulated whole saliva 23.6
	Cuida *et al*[121]	Healthy subjects Parotis saliva 3.2 Stimulated whole saliva 22.0 Mucosal transudate 40.9
	Müller *et al*[111]	HIV infected with or without oral candidiasis Parotis 0.06–0.41
Urine	Holt *et al*[120]	Infants and children (range) Controls 24 µg/l (5–650) Cystitis 182 µg/l (18–992) Pyelonephritis 1000 µg/ml (360–7000)
Faeces	Røseth *et al*[19]	Median (range) Healthy subjects 2 (0.5–8) Controls 10.5 (1.1–80) Crohn's disease 43 (8–2000) Ulcerative colitis 19.5 (2.4–866.4)
	Røseth *et al*[17]	Colorectal cancer 50.0 (4.5–950)
	Gilbert *et al*[102]	Healthy controls, range 0–9 Colorectal cancers (mean (SD)) Right side 55.1 (58.9) Left side 79.3 (58.2)
	Meling *et al*[108]	Mean (95% confidence intervals) Normal baseline 4.9 (1.5–15.6) NSAID treated 9.0 (6–27)
Synovial fluid	Berntzen *et al*[14]	Median (range) Rheumatoid arthritis 18 (2–375) Osteoarthritis 0.9 (0.2–2)
Empyema fluid (supernatant)	Santanagopalan *et al*[9]	Not quantitated. Calprotectin functional assay for *C albicans* growth inhibition

* Concentrations in cerebrospinal fluid and urine given as µg/l, all others given as mg/l.

Such values are found regularly in plasma from citrated blood stored for 14–21 days.

Increased plasma calprotectin concentrations are found regularly in HIV infected individuals, and a strong further increase in response to zidovudine treatment seems to predict a favourable prognosis.[119] Curiously, increased plasma calprotectin is associated with a poor chance of survival in patients with alcoholic liver disease irrespective of the degree of parenchymal liver damage.[127]

During surgery, complement activation can be demonstrated by increased concentrations of the terminal complement complex or C3b in plasma. Simultaneously, granulocyte lysosomal proteins (myeloperoxidase and lactoferrin) and calprotectin increase, but the latter remains elevated many hours longer than the former, even after the complement activation signals have returned to baseline.[116 128 129] Coronary sequestration of both granulocytes and calprotectin was demonstrated in the early reperfusion period after cardiac surgery and cold cardioplegic arrest.[114]

Recently, Arvesen *et al*[112] found that plasma calprotectin concentrations are increased in patients with coronary artery disease. Even patients with unstable angina or small (non-Q) myocardial infarctions had mean values about three times the upper reference limit. An interesting possibility is that the elevations may reflect an increased (inherited or acquired?) myelomonocytic response to even minor stimuli that in the long run may predispose to atherosclerosis.

Plasma calprotectin levels are increased in active rheumatic diseases; Berntzen *et al*[12-14] found 1–46 mg/l in rheumatoid arthritis

Table 7 Inflammation markers in meningococcal infections

	Calprotectin		*CRP*		*Leukocytes*		*LPS*		*IL-6*	
Clinical presentation	*mg/l*	*range*	*mg/l*	*range*	*x E9/l*	*range*	*ng/l*	*range*	*µg/l*	*range*
Fulminant sepsis*	13.8	10.5–119.9	85	19–225	7.1	2.2–33.3	2800	210–170000	550	55–2400
Meningitis*	6.8	2.9–51.0	137	24–328	22.6	3.8–30.9	<3	<3–210	0	0–297
Mild syst menin*	6.7	1.6–14.3	75	16–162	14.6	9.5–35.0	45	<3–600	3	0–102
Reference value†	0.7	0.3–1.6	≤10				<3		<0.05	

*Fulminant sepsis plasma (n=13) measured 12 h or less after onset of symptoms. Meningitis plasma (n=20) measured 24 h or less after onset. Mild systemic meningococcal (syst menin) disease plasma (n=6) measured at variable time intervals after onset of symptoms. Median value and range is given for each parameter.
† Reference values are from Golden *et al*[97] for calprotectin, Waage *et al*[124] for lipopolysaccharide (LPS) and interleukin (IL)-6, and Hjortdal *et al*[125] for C reactive protein (CRP). See also Brandtzæg and Kierulf.[23]

patients and 2–24 mg/l in juvenile rheumatoid arthritis, while Haga *et al*[96] reported a mean value of 3.6 mg/l in systemic lupus erythematosus patients compared with 1.05 mg/l in matched controls. In both rheumatoid arthritis and systemic lupus erythematosus, plasma calprotectin values appear to be an objective parameter for the assessment of disease activity and response to treatment. Furthermore, both plasma and synovial fluid calprotectin clearly distinguish rheumatoid from osteoarthritis.[14]

Plasma calprotectin concentrations are increased in endogenous posterior uveitis.[95] Patients may also have raised antineutrophilic cytoplasmatic antibodies (ANCA), but the ANCA titres did not correlate with calprotectin levels. The authors suggested the latter may be a sensitive indicator of disease activity for endogenous posterior uveitis. There seems to be a similar situation for cystic fibrosis.[97]

In a series of patients with newly diagnosed pulmonary cancers,[123] increased plasma calprotectin was found in 81%, while other disease indicating parameters lagged behind: CRP, 45%; orosomucoid, 48%; haptoglobulin, 59%; α_1 antitrypsin, 31%; alanine aminotransferase, 5%; aspartate aminotransferase, 0%; γ glutamyl transferase, 27%; alkaline phosphatase, 10%. Strikingly, about 15% of the patients had calprotectin concentrations (and other markers) close to or below the upper reference limit despite advanced, invasive disease. This raises the possibility that some tumours may release substances capable of inhibiting the emigration or activation of myelomonocytic cells. At least such cells would be expected to participate in the removal of dead tumour cells.

CALPROTECTIN IN CEREBROSPINAL FLUID

Limited data have been published, but calprotectin levels in CSF seem to distinguish between HIV encephalitis and opportunistic infections in the central nervous system in HIV infected patients.[18]

CALPROTECTIN IN ORAL FLUIDS

Concentrations between 3 and 40 mg/l have been measured in different oral secretions (table 6). Müller *et al*[111] found lower calprotectin concentrations in HIV infected patients who suffered from oral candidiasis compared with all HIV infected subjects, and with healthy controls.

CALPROTECTIN IN URINE

Increased calprotectin is found in patients with urinary tract infections, particularly in patients with renal involvement.[120] Calprotectin measurement has been suggested as a urinary marker of asthma.[130]

CALPROTECTIN AND GASTROINTESTINAL DISEASES

Røseth *et al*[19] developed a method for the determination of calprotectin in stool as an alternative to α_1 antitrypsin for the evaluation of disease activity in inflammatory bowel disease (IBD). It was, however, soon realised that increased faecal calprotectin is a marker of diseases of the gastrointestinal tract, including gastric cancer, colorectal adenoma or cancer, Crohn's disease, and ulcerative colitis.[17 19 101 105] The method depends on the preparation of a simple buffer extract of a small spot sample of stool, and quantitation of calprotectin by enzyme linked immunosorbent assay (ELISA). Only a fraction of the total calprotectin content in the stool sample is brought into solution, and most of it is found in high molecular size complexes (Røseth and Fagerhol, 1992, unpublished).

In recently diagnosed colorectal cancers, stool calprotectin concentrations above an upper reference limit of 10 mg/l were found in more than 90% of the patients.[19] In this study the value decreased below 10 mg/l after successful radical resection. No significant differences in calprotectin were found in cancers of Duke's stages A and B compared with C and D, which suggests that a positive calprotectin test may be found even in early cancers. This is supported by the finding of increased concentrations in more than 60% of patients with colorectal adenoma irrespective of size and location.[101] Again, calprotectin concentrations returned to normal after removal of the adenomas.[101] Although calprotectin is also synthesised by squamous epithelial cells, it is not found in epithelial cells of the bowel nor in colorectal cancer cells. The numbers of neutrophils, however, are increased significantly in the peritumoral stroma in colonic carcinomas.[131] The working hypothesis is that different types of pathological changes in the gastrointestinal tract cause increased permeability of the mucosa leading to increased migration of granulocytes and monocytes towards chemotactic substances in the gut lumen. An important factor for the high sensitivity of the faecal calprotectin test is the fact that this protein is remarkably resistant to proteolysis in the presence of calcium.[2] This may explain why the test has been found positive in more than 90% of patients with gastric cancer.[19]

It is notable that faecal calprotectin shedding may be caused by drugs[108] or other inflammatory reactions in the gastrointestinal system. Thus, extensive clinical studies are being conducted in this area, particularly aimed at confirming a negative predictive value for colorectal cancer.[17] Haemoglobin or faecal occult blood are used extensively as markers in screening for colorectal cancer. Calprotectin is a less variable parameter than haemoglobin in faecal samples from patients with colorectal cancers,[102] and although both parameters are elevated significantly, they do not correlate. Thus the mechanism of luminal calprotectin entry appears to be both different from and less erratic than bleeding.[102]

In patients with active IBD, faecal calprotectin concentrations above 100 mg/l are regularly found,[101] and a diagnosis of IBD should not be made in a symptomatic patient with a faecal calprotectin level below 10 mg/l. Highly significant correlations were found between this test and the IBD disease activity determined by endoscopy, histology or excretion of ^{111}indium labelled autologous granulocytes.[101 105] The latter method has been regarded as the "gold standard" but its use is limited by complexity, cost, and exposure of the patient to irradiation. The relatively simple faecal calprotectin test is an attractive alternative as it can be repeated at any time and, because of the stability of calprotectin, samples can be mailed by the patient to the laboratory. Treatment response in patients with Crohn's disease can be monitored by faecal calprotectin measurements.

CONCLUSION

The clinical relevance of calprotectin seems to be related to its physiological functions during homeostasis as well as in pathogenesis. These functions are intracellular as well as extracellular. Disease modulation during rheumatoid arthritis and possible antitumour effects of calprotectin may result from specific mechanisms other than antimicrobial activity. Calprotectin is a non-specific marker for activation of granulocytes and mononuclear phagocytes. It is released from these cells and is thus expected to be found in secretory and excretory products in different parts of the body.

Possible clinical applications

The diagnostic value of calprotectin has been emphasised in several publications (tables 5 and 6) that recommend calprotectin as an inflammatory disease marker. Extensive and systematic clinical documentation is still lacking, however, some larger studies are ongoing. RIA[33] and ELISA[15] have been established as immunoassays for calprotectin measurement in body fluids, and are also applied to stool samples.[17 19] Two commercial ELISA assays exist, with an analytical sensitivity at the ng/ml level. In the NycoCard (Nycomed, Oslo, Norway) immunoassay format[125 132] a prototype quantitative rapid test for calprotectin has been established with an analytical sensitivity of approximately 0.05 μg/ml. Documentation will increase as commercial calprotectin tests become easier and more widely available. Immunohistochemical staining for calprotectin may be a diagnostic tool in inflammatory skin diseases, malignant conditions, and a variety of other diseases.[3 133] Several commercial and non-commercial monoclonal antibodies are available (table 2).

Monitoring of disease activity and therapy may be relevant in inflammatory diseases such as rheumatoid arthritis, cystic fibrosis, Crohn's disease, asthma,[94 97 130] and others (tables 5 and 6).

Therapeutic use of calprotectin (or some of its structural elements) is a fascinating thought with its protective biological properties. Lehrer[32] calls it "an internal ointment that restricts the growth of *C albicans* on nearby skin." Could it be used as an external ointment? In terms of therapy could the molecule be used in rheumatic, inflammatory, infectious or malignant diseases?

Unpublished data

SYNTHESIS AND RELEASE OF CALPROTECTIN FROM HUMAN MONOCYTES IN VITRO

A series of experiments were performed immediately after the discovery of calprotectin, to gather data concerning the mechanisms involved in the release of calprotectin from monocytes. These data have not previously been published, nor have similar data been published by others. We have therefore included them in the present review.

Materials and methods

Human monocytes were isolated from healthy fasting donors as described previously[134 135] and cultured in RPMI 1640 (Gibco-Biocult, Paisley, Scotland) containing 20% inactivated fetal calf serum (Flow, Irvine, Scotland). The final cell preparations consisted of more than 95% monocytes demonstrated by differential counting, phagocytosis and staining for non-specific esterase.[136] The presence of calprotectin was determined by single radial diffusion or ELISA after incubation periods of up to 46 hours. The synthesis of calprotectin was studied by addition of ^{35}S-methionine to the medium, and radiolabelled calprotectin was determined by an immunoaffinity column with immobilised, monospecific rabbit anti-calprotectin antibodies.

The following compounds were added to monocyte cultures: immune complexes formed by incubating equivalent amounts of human serum albumin or transferrin (Sigma, St Louis, Missouri, USA) and corresponding antibodies (Dakopatts, Copenhagen, Denmark); aggregated human IgG (Gammaglobulin, Kabi, Stockholm, Sweden) prepared by heating a 1% solution in 0.15 M NaCl at 63°C for 12 minutes, and precipitation with Na_2SO_4 (67 g/l); cross-linked human immunoglobulins prepared by reacting IgG (10 g/l) with 0.5% glutaraldehyde in 0.1 M acetate buffer, pH 4.0 for one hour at room temperature, followed by dialysis against distilled water and phosphate buffered saline, pH 7.35; concanavalin A (ConA) and wheat germ agglutinin (WGA) (Sigma); phytohaemaglutinin (PHA) (Wellcome, Beckenham, Kent, UK); ionophores A 23187 and nigericin

Table 8 Release of calprotectin from human monocytes after 16 hours in vitro culture

Stimulant	Concentration µg/ml	Serum* 20%	No. of cultures	Calprotectin release† (ng/ml)
Lectins				
ConA	25	+	14	< 25
ConA	25	−	2	98
PHA	25	+	8	49
PHA	25	−	2	130
WGA	25	+	4	46
WGA	25	−	2	1050
Antigen/antibody complexes				
HSA/anti-HSA	10‡	+	8	665
HSA/anti-HSA	10‡	−	2	265
Transferrin/antitransferrin	2‡	+	6	440
Heat aggregated IgG (IgG-H)	700	+	2	15
Glutaraldehyde aggregated IgG (IgG-G)	700	+	2	25
HSA/anti-HSA + IgG-H	10‡ + 700	+	2	48
HSA/anti-HSA + IgG-H	10‡ + 250	+	2	52
HSA/anti-HSA + IgG-G	10‡ + 700	+	2	37
HSA/anti-HSA + IgG-G	10‡ + 250	+	2	49
Ionophores				
A 23187	0,25	+	14	165
A 23187	0,25	−	6	319
Nigericin	2,5	+	2	> 1000
Endotoxin	25	+	12	136
Phorbol ester				
TPA	1	+	2	2238

* Fetal calf serum inactivated at 56°C for 30 min.
† The release from unstimulated control cells was < 25 ng/ml.
‡ Amount of antigen present in the immune complex added.
HSA, human serum albumin.

Table 9 Release of lysozyme and β glucuronidase from stimulated human monocytes in vitro

Stimulant*	Lysozyme % of unstimulated control (SEM)	Cultures (n)	β glucuronidase % of unstimulated control (SEM)	Cultures (n)
Immune complexes	695 (189)	12	610 (79)	8
Endotoxin	138 (45)	10	107 (4)	8
A 23187	435 (251)	12	198 (68)	10
Lectins	92 (8)	16	97 (21)	14
Nigericin	257	2	356	2
TPA	664 (114)	10	425 (70)	10

* The final concentrations of stimulants were as given in table 8.

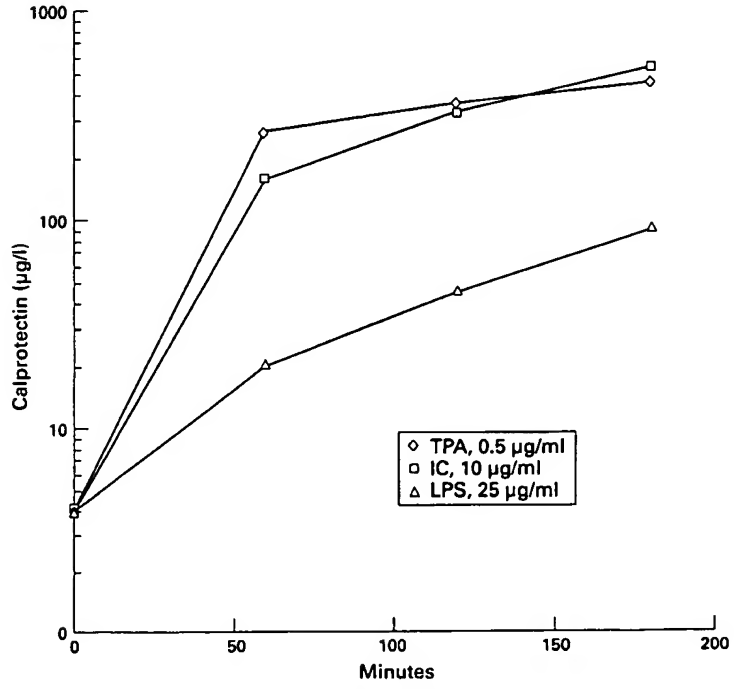

Figure 1 Time course for release of calprotectin from human monocytes in vitro. TPA, 12-O-tetradecanoylphorbol-13-acetate; LPS, endotoxin; IC, immune complexes. The final concentrations in µg/ml of the added substances are indicated.

(Lilly Research Corporation, Indianapolis, Indiana, USA); endotoxins *E coli* 0111:B4 and *E coli* 055:B5 (Difco, Detroit, Michigan, USA); actinomycin D (Serva, Heidelberg, Germany); 12-O-tetradecanoylphorbol-13-acetate (TPA) (PL Biochemicals, Milwakee, Wisconsin, USA); cycloheximide, cytochalasin B, and prostaglandin E_1 (PGE_1) (Sigma).

Lactate dehydrogenase, lysozyme, and β glucoronidase activities were determined in culture supernatants and cell homogenates.[137-139]

Results

Freshly prepared monocytes contained 2.5 ± 0.7 pg calprotectin per cell, and after incubation for 9–10 hours the content was 2.3 ± 0.2 pg/cell.

Various agents known to interact with and activate[140] monocytes were added to monocyte cultures. None of these, except TPA at 1 µg/ml caused cytotoxicity (increased trypan blue uptake) within the time period studied. As shown in table 8, very small amounts of calprotectin were released from monocytes cultured for 16 hours in the presence of lectins and 20% inactivated fetal calf serum. In the absence of serum slightly higher amounts (but less than 1% of total cellular calprotectin content) were released after ConA or PHA stimulation, while WGA caused a noticeably higher release. No release of lactate dehydrogenase or β glucuronidase, and no increased lysozyme release was seen in lectin stimulated cultures (table 9). Immune complexes led to a marked release of calprotectin over two to three hours (fig 1), and about 8% of the calculated total cellular calprotectin was released. This release was two to threefold higher in the presence of fetal calf serum (table 8). Native serum was 25% more active than inactivated serum, and native serum alone promoted the release of calprotectin in the absence of immune complexes. Addition of immune complexes induced a marked release of both β glucuronidase and lysozyme (table 9), but essentially no release of lactate dehydrogenase.

Phorbol ester (TPA) at 500 ng/ml caused a quite rapid and marked release of calprotectin (about 7% of total cellular content), but no signs of cytotoxicity in three hours (fig 1). Release of the total intracellular calprotectin was seen when a cytotoxic dose of TPA (1 µg/ml) was applied for 14 hours (table 8). The divalent ionophore A23187 caused a moderate release of calprotectin, and the monovalent ionophore nigericin a substantial release (table 8). Both ionophores increased moderately the release of β glucuronidase and lysozyme (table 9).

Endotoxin induced a moderate but distinct release of calprotectin (table 8), but no significant increase in lysozyme or β glucuronidase secretion (table 9). A very small amount (less than 2% of total cellular content) was released during the first three hours (fig 1).

To learn more about the mechanism of calprotectin release, addition of several other compounds were tested. Prostaglandin E_1 (10 µM) did not influence the release of calprotectin during incubation with TPA. The

Table 10 Release of calprotectin, lysozyme, and β glucuronidase from stimulated human monocytes in vitro, with potentially interfering substances added

Stimulant*	Additions†	Calprotectin‡	Lysozyme‡	β glucuronidase‡
Immune complexes	Dexamethasone	101 (7)	84 (7)	87 (35)
	Promethazine	98	131	134
	Indomethacin	81 (12)	100 (5)	115 (8)
Endotoxin	Dexamethasone	44 (6)	99 (15)	141
	Promethazine	248 (70)	1250	597 (260)
A 23187	Dexamethasone	122	93 (7)	18
	Promethazine	311	452	426
	Indomethacin	196 (12)		
Lectins	Dexamethasone	95 (25)	101 (18)	58 (15)
	Indomethacin			230

* The final concentrations of stimulants were as given in table 8.
† Final concentrations: dexamethasone, 10 μg/ml; promethazine and indomethacin; 0.1 mM.
‡ Each value is the mean of 2–8 cultures, and calculated as % of stimulated control (SEM).

prostaglandin synthesis inhibitors indomethacin and acetylsalicylic acid had no effect on the release caused by immune complexes or lectins (table 10). Release induced by A23187 was enchanced twofold by indomethacin but not by acetylsalicylic acid. As indomethacin has other effects besides blocking prostaglandin synthesis,[141] our tentative conclusion is that products of the prostaglandin pathway are not involved in calprotectin secretion. The antihistamine promethazine enhanced the release of calprotectin induced by endotoxin, A23187 or TPA (data not shown), whereas the release induced by immune complexes was unchanged (table 10). Similarly, both lysozyme and β glucuronidase release was enchanced by addition of promethazine to A23187 or endotoxins, but not to immune complex stimulated monocytes (table 9). Dexamethasone inhibited calprotectin release induced by endotoxins but not that induced by A23187, lectins or immune complexes. This drug had no effect on the lysozyme release, but the release of β glucuronidase induced by A23187 and lectins was inhibited. Cytochalasin B (10 μg/ml), which interferes with actin polymerisation, had no effect on calprotectin release induced by endotoxin, but a twofold enhancing effect in the presence of PHA.

Dexamethasone (10 μg/ml), dibutyryl cAMP (1 mM), cAMP (1 mM), verapamil (25 μg/ml), acetylsalicylic acid (0.1 mM), and purified protein derivative of Bacillus Calmette-Guerin (PPD) had no effect of calprotectin release from unstimulated cells.

Discussion

The release of calprotectin from isolated monocytes can result from general cytotoxicity. If sufficiently high doses are used—for example, TPA at 1 μg/ml, the total calprotectin pool may be released. However, partial release was observed without concomitant lactate dehydrogenase release or trypan blue uptake. This suggests that either calprotectin release is a very sensitive indicator of cytotoxicity or the release is caused by other mechanisms.

There was no definite evidence for involvement of the prostaglandin system in calprotectin release, and the fraction released during three to four hours never exceeded 8% of the total cellular pool.

The agents that promoted lysosomal enzyme release most (immune complexes, TPA, ionophores) also increased the calprotectin release, although not exactly in the same proportion. A correlation was observed between lysozyme and calprotectin release. The effects of dexamethasone served to distinguish these processes in that it reduced calprotectin release while leaving lysozyme and β glucuronidase release unchanged in response to endotoxin. In the presence of lectins or A23187 the opposite was found: dexamethasone reduced β glucuronidase release whereas calprotectin and lysozyme release were unchanged. It is therefore likely that calprotectin release is different from the lysozyme and lysosomal release processes. Clearly, calprotectin release can be caused by a number of membrane perturbing agents, and calprotectin is either derived from the plasma membrane or from an intracellular compartment by some special release process.[142]

Anti-calprotectin antibodies added to human monocytes in culture induced the synthesis of the protein component of thromboplastin,[140] which suggests that calprotectin is at least in part located in the plasma membrane, as observed by Dale *et al*[75] and Guignard *et al*.[88]

Conclusions

Calprotectin is fascinating because it holds unrevealed secrets. We know that calprotectin is an important granulocyte marker and a multifunctional regulatory protein in inflammatory processes. There is more work to do in order to understand fully basic mechanisms such as quaternary structure, complex assembly, molecular functions, and biological effect mechanisms.

Much has been reported regarding the clinical relevance and diagnostic potential of calprotectin and its subunits, and larger clinical studies are continuing. Further work may gather speed as commercial kits for calprotectin measurement become more readily available. Interesting therapeutic concepts may be found for calprotectin, as the relation of the structure and function of the molecule are better understood.

In the meantime we will do as Lehrer[32] recommended in 1993: "Stay tuned. Calprotectin, whatever it is, could be interesting."

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Novel Clinical Markers of Vascular Wall Inflammation

Gavin J. Blake and Paul M. Ridker

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Anti-Inflammatory Mechanisms in the Vascular Wall

Clinical Imaging of the High-Risk or Vulnerable Atherosclerotic Plaque

Novel Clinical Markers of Vascular Wall Inflammation

CD40 Signaling and Plaque Instability

Innate and Adaptive Immune Mechanisms in Atherosclerosis

Andreas Zeiher, Guest Editor

Novel Clinical Markers of Vascular Wall Inflammation

Gavin J. Blake, Paul M. Ridker

Abstract—Inflammatory processes play a pivotal role in the pathogenesis of atherosclerosis and mediate many of the stages of atheroma development from initial leukocyte recruitment to eventual rupture of the unstable atherosclerotic plaque. Elevated plasma levels of several markers of the inflammatory cascade have been shown to predict future risk of plaque rupture. These markers include P-selectin, interleukin-6, tumor necrosis factor-α, soluble intercellular adhesion molecule-1, and C-reactive protein (CRP). Produced in the liver in response to interleukin-6, CRP has emerged as the most powerful inflammatory marker of future cardiovascular risk. Initially considered an innocent bystander in the atherosclerotic process, recent evidence suggests that CRP may have direct proinflammatory effects. Numerous large-scale, prospective studies have found that elevated baseline levels of CRP are a strong independent predictor of future vascular risk. Furthermore, aspirin and statin therapy appear to be particularly effective among individuals with high CRP levels. The addition of CRP screening to traditional lipid testing has the potential to identify individuals at high risk for future cardiovascular events who may benefit from targeted preventive interventions. **(*Circ Res.* 2001;89: 763-771.)**

Key Words: inflammation ■ risk factors ■ atherosclerosis

From the initial phases of leukocyte recruitment, to eventual rupture of vulnerable atherosclerotic plaque, inflammatory mediators appear to play a key role in the pathogenesis of atherosclerosis.[1] Early atherosclerotic lesion development involves tethering and adherence of monocytes to, and subsequent transmigration through, the vascular endothelium. Differentiation of monocytes to macrophages and subsequent accumulation of lipid results in foam cell generation and fatty streak formation. Further recruitment of inflammatory cells and proliferation of smooth muscle cells lead to the development of a mature atherosclerotic plaque, with a fibrous cap separating the prothrombotic lipid pool from luminal blood flow. Fibrous cap thinning may lead to plaque rupture and precipitate the onset of an acute ischemic event.[2] Accumulating evidence suggests that inflammatory processes are intimately involved in each of these stages in atherogenesis.

Inflammatory Mechanisms in Atherothrombosis

The adherence and subsequent transmigration of leukocytes across the vascular endothelium are mediated by cellular adhesion molecules (CAMs).[3] The selectins are adhesion molecules that mediate the initial rolling of inflammatory cells along endothelial cells and platelets. P-selectin is stored in the α granules of platelets and the Weibel-Palade bodies of endothelial cells[4] and can be rapidly redistributed to the surface of these cells after stimulation by agonists such as thrombin and ADP. E-selectin is synthesized de novo by

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endothelial cells when activated by interleukin-1 (IL-1) or tumor necrosis factor-α (TNF-α).[5]

Available evidence suggests a role for the selectins in the early stages of atherogenesis. P-selectin expression has been shown to precede macrophage and lymphocyte accumulation in rabbits fed a high-cholesterol diet.[6] E-selectin and P-selectin are preferentially expressed in the endothelium overlying atherosclerotic plaques,[7] whereas administration of anti–P-selectin antibodies results in reduced monocyte rolling and attachment to carotid vascular endothelium.[8] Furthermore, P-selectin–deficient mice show a complete absence of leukocyte rolling and develop reduced atheromatous lesions[9] and are protected from neointimal hyperplasia after vascular injury,[10] suggesting that blockade of P-selectin may be a potential therapeutic strategy to decrease restenosis.

Intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) belong to the immunoglobulin superfamily of CAMs. These adhesion molecules are thought to regulate attachment and transendothelial migration of leukocytes.[11] Both macrophages and endothelial cells produce ICAM-1 in response to inflammatory cytokines such as IL-1, TNF-α, and interferon-γ, whereas VCAM-1 expression is mainly restricted to endothelial cells.[7] Cytokine-stimulated endothelial cells also produce monocyte chemotactic protein-1 (MCP-1), monocyte colony-stimulating factor, and IL-6, which further amplifies the inflammatory cascade.[12] VCAM-1 expression has been demonstrated to precede macrophage and T-lymphocyte recruitment to atheromatous plaque,[6] and rabbits fed a high-cholesterol diet express VCAM-1 on the endothelium of aortic plaque.[13] ICAM-1 expression by endothelial cells has been demonstrated over all types of atheromatous plaque.[7]

TNF-α is a pleiotropic cytokine produced by a variety of cells including macrophages, endothelial cells, and smooth muscle cells.[14,15] TNF-α, along with interferon-γ and IL-1, stimulates IL-6 production by smooth muscle cells.[16–18] IL-6 gene transcripts are expressed in human atheromatous lesions,[19,20] and IL-6 is the main hepatic stimulus for C-reactive protein (CRP) production.[21]

CRP is an acute-phase reactant that serves as a pattern-recognition molecule in the innate immune system.[22] CRP has been traditionally thought of as a bystander marker of vascular inflammation, without playing a direct role in the inflammatory process. However, recent evidence suggests that CRP may contribute directly to the proinflammatory state. CRP stimulates monocyte release of inflammatory cytokines such as IL-1b, IL-6, and TNF-α[23] and may also directly act as a proinflammatory stimulus to phagocytic cells by binding to the FcγRII receptor.[24] It has also been recently demonstrated that CRP causes expression of ICAM-1 and VCAM-1 by endothelial cells[25] and mediates MCP-1 induction in endothelial cells, an effect that is inhibited by simvastatin and fenofibrate.[26] CRP opsonization of low-density lipoprotein (LDL) also mediates LDL uptake by macrophages.[27]

CRP has been localized directly within atheromatous plaque where it precedes and mediates monocyte recruitment.[28] CRP is an activator of complement, and it has been shown to colocalize with the membrane attack complex in early atherosclerotic lesions.[29] The principal source of CRP production is the liver. However, recent data show that arterial tissue can produce CRP as well as complement proteins. These products and their associated mRNA are substantially upregulated in atherosclerotic plaque, with smooth muscle cells and macrophages the main producers.[30] This supports the concept that CRP may be an endogenous activator of complement in atheromatous lesions.

As the atheromatous plaque matures, it develops a fibrous cap and lipid core. The vulnerable plaque is characterized by a thin fibrous cap and large lipid pool.[2] Interstitial collagen, produced by smooth muscle cells, confers tensile strength and stability to the fibrous cap. Platelet-derived growth factor, released by platelets during thrombosis, and transforming growth factor-β, increase the rate of collagen production. Conversely, macrophages, in response to stimulation by T cells, can produce matrix metalloproteinases (MMPs), which actively break down the collagen and other extracellular matrix proteins in the fibrous cap. T cells may also produce interferon-γ, which signals to smooth muscle cells to decrease collagen synthesis.[31] Thus a dynamic balance is maintained between collagen synthesis and breakdown. If the equilibrium is tipped to a proinflammatory state, fibrous cap thinning and eventual rupture may result.

Rupture of the fibrous cap results in spilling of the prothrombotic lipid pool into the lumen, likely heralding an acute ischemic event. Tissue factor, overexpressed by endothelial cells and macrophages, is a key initiator of thrombosis. IL-1 and TNF-α stimulate tissue factor expression by endothelial cells, and it has recently been shown that a CD40 ligand (CD154) binding to leukocytes can stimulate tissue factor expression.[32] Platelets express CD154, illustrating a potentially important interaction between proinflammatory and prothrombotic pathways.[33] Moreover, it has recently been shown that leukocyte binding and migration across a carpet of platelets adherent to diseased or injured intima are dependent on the leukocyte integrin Mac-1 and platelet glycoprotein 1bα.[34]

The trigger for the inflammatory response, however, remains unclear. Attention has focused on infectious sources as potential instigators. Distant infection with organisms such as *Helicobacter pylori*, leading to increased circulating cytokines, or persistent local infectious processes within atherosclerotic plaque by intracellular organisms such as *Chlamydia pneumoniae* and cytomegalovirus, could potentially be the stimulus. Although pathophysiologically appealing, data regarding the value of testing for antibodies to these and other infectious agents in prospective epidemiological studies of cardiovascular risk are inconclusive.[35–43]

The inflammatory response may be promoted at several different sites. Although many inflammatory markers are derived from the liver, including CRP, fibrinogen, and serum amyloid A, low levels may also be derived from other sources including the endothelium itself. Production of inflammatory markers is stimulated by circulating cytokines such as IL-6 and TNF-α, which in turn may also be generated from a variety of systemic sources, including adipose tissue, which is a potent source of cytokines, and inflammatory cells either in the atherosclerotic lesion in the arterial wall or elsewhere.

Inflammation and Endothelial Dysfunction

Several workers have sought to explore the hypothesis that impairment of endothelial function by inflammatory responses might provide a link between systemic inflammation and ischemic syndromes. In this regard, the administration of *Salmonella typhi* vaccine, used to generate a systemic inflammatory response in healthy volunteers, was associated with a temporary but profound dysfunction of arterial endothelium, as assessed by forearm blood flow response to acetylcholine and bradykinin.[44] Furthermore, among patients with coronary artery disease, increased CRP levels have been shown to be associated with impairment of forearm endothelial vascular reactivity.[45] Importantly, normalization of CRP levels over time was associated with a significant improvement in endothelial responses. Recent work has also demonstrated that CRP levels are inversely related to basal endothelial nitric oxide synthesis.[46] These data suggest that endothelial dysfunction may be an important factor in the relationship between low-grade chronic inflammation and cardiovascular disease. Interestingly, statin therapy in humans has been shown to upregulate endothelial nitric oxide synthesis[47] and improve endothelial-dependent coronary vasodilation after 1 month of treatment.[48]

Antiinflammatory Effects of Lipid Lowering

Lipid lowering has been found to have favorable effects on inflammatory processes within atheromatous plaque. Rabbits fed a high-cholesterol diet develop atheroma with a high number of macrophages in the lipid pool.[49] These macrophages overexpress MMP-1, the rate-limiting enzyme in collagen breakdown. If the rabbits were switched to a low-cholesterol diet, the numbers of macrophages and levels of MMP-1 were dramatically reduced.

Statin therapy has also been shown to have salutary effects on plaque composition. Fluvastatin and lovastatin decrease MMP-1 expression in human vascular endothelial cells in a time- and dose-dependent manner.[50] Fluvastatin and pravastatin have recently been shown to decrease MMP-1, MMP-3, and MMP-9 expression by macrophages in the intima of hyperlipidemic rabbits and to increase procollagen production by smooth muscle cells.[51] Pravastatin causes favorable changes in atheromatous plaque independent of its cholesterol-lowering effects. Pravastatin-treated monkeys had better vasodilator function and fewer macrophages in the intima and media and less calcification and less neovascularization in the intima than control animals with similar changes in lipid profile caused by diet alone.[52] Recent data from human carotid plaque show favorable change with pravastatin therapy, with fewer macrophages, reduced MMP-2 activity, and higher collagen content.[53]

Statins also cause decreased macrophage expression of soluble ICAM-1 (sICAM-1) and lipopolysaccharide-induced secretion of IL-6 and TNF-α.[54–56] Simvastatin reduces monocyte expression of TNF-α and IL-1b,[57] whereas atorvastatin reduces MCP-1 levels in the intima and media of hypercholesterolemic rabbits.[58] This effect is related to a reduction in nuclear factor-κB activation, a transcription factor involved in the induction of other proinflammatory cytokines, such as IL-1 and TNF-α,[59] and the regulation of E-selectin expression.[60] Lipophilic statins have also been shown to decrease tissue factor expression and activity in cultured human monocyte-derived macrophages.[61] As will be described later, in clinical studies statins have been found both to attenuate inflammatory risk and to reduce CRP levels.[62–67]

Statins also directly inhibit induction of major histocompatibility complex class-II (MHC-II) expression by interferon-γ and thus act as repressors of MHC-II–mediated T-cell activation.[68] This effect is due to inhibition of the transactivator CIITA and is observed in several cell types including endothelial cells and macrophages. Recent studies also suggest that statins may promote vasculogenesis by mobilizing bone marrow–derived endothelial precursor cells and augmenting circulating endothelial precursor cells through stimulation of the Akt signaling pathway.[69,70]

Inflammatory Markers for Clinical Risk Prediction

Overt hyperlipidemia is present in less than half of all patients who have myocardial infarction. Given the pivotal role of inflammatory mediators in atherogenesis and the determination of plaque vulnerability, attention has focused on whether plasma levels of inflammatory markers can help predict individuals at increased risk for plaque rupture.[71] Candidate markers include P-selectin, sICAM-1, IL-6, TNF-α, and CRP.

Given their role in the initial stages of atherogenesis, researchers have investigated the value of CAMs for prediction of subsequent vascular risk among healthy subjects. After activation by cytokines, CAMs are shed from the surface of endothelial cells and leukocytes and plasma levels of circulating CAMs can be measured. Although the pathogenic role of these circulating CAMs remains unclear, these molecules may serve as markers of endothelial activation and vascular inflammation.

Soluble P-selectin has been shown to be an independent predictor of future cardiovascular risk in a large-scale prospective study of 28 263 apparently healthy women enrolled in the Women's Health Study (WHS).[72] Overall mean levels of P-selectin were significantly higher at baseline among women who subsequently experienced cardiovascular events compared with those who did not. The risk of future cardiovascular events among women in the highest quartile of P-selectin levels was 2.2 times higher than those in the lowest quartile ($P=0.01$), an effect that was independent of traditional risk factors.

In a prospective study among 14 916 healthy men enrolled in the Physicians' Health Study (PHS), baseline levels of sICAM-1 have also been shown to be independent predictors of future cardiovascular risk.[73] Baseline levels were higher among men who subsequently developed myocardial infarction than those who did not, and the adjusted relative risk for those with the highest quartile of baseline sICAM-1 levels compared with those in the lowest quartile was 1.8 ($P=0.03$). The risk of myocardial infarction associated with raised concentrations of sICAM-1 seemed to increase with length of follow-up, an effect potentially consistent with the early role of ICAM-1 in the atherosclerotic process.

The Atherosclerosis Risk in Communities (ARIC) study also found that sICAM-1 was an independent predictor of

coronary heart disease.[74] However, in both the PHS and the ARIC study, baseline plasma levels of VCAM-1 were not associated with an increase in cardiovascular risk.[74,75] These data suggest that there may be important distinctions between the roles of different CAMs in atherogenesis. Indeed, in human atheroma, ICAM-1 is highly expressed by both endothelial cells and macrophages whereas VCAM-1 is found in fewer than one third of lesions and its expression is predominantly restricted to endothelial cells and occasional spindle cells.[7]

IL-6 and TNF-α occupy a central role in the amplification of the inflammatory cascade. Evidence in support of the role of inflammatory processes in acute coronary syndromes comes from data showing that increased levels of IL-6 and IL-1 receptor antagonist at 48 hours after admission are associated with a complicated in-hospital course.[76] Furthermore, elevations of TNF-α in the stable phase after myocardial infarction were associated with an increased risk of recurrent coronary events.[77] Specifically, those with baseline levels in excess of the 95th percentile of the control distribution had a 2.7-fold increase in risk (P=0.004). The plasma half-life of TNF-α is short, a factor that may limit its potential clinical utility as a screening tool.

In a further analysis from the PHS, baseline levels of IL-6 were higher among apparently healthy men who subsequently had a myocardial infarction than among those who did not.[78] The risk of future myocardial infarction increased with increasing quartiles of baseline IL-6 concentration such that the men in the highest quartile at entry had a relative risk 2.3 times higher than those in the lowest quartile (P=0.005). CRP was the strongest correlate of IL-6 in these data (r=0.43; P<0.001), an observation consistent with the finding that IL-6 is the main stimulus for hepatic production of CRP.[21] Nevertheless, the relationship of IL-6 with subsequent risk remained after adjustment for CRP.

Of all the plasma markers of vascular inflammation, CRP has been the most extensively investigated in clinical studies. Baseline levels of CRP are a strong independent predictor of risk of future myocardial infarction, stroke, peripheral vascular disease, and stroke and vascular death among healthy individuals without known vascular disease.[79–89] Furthermore, levels of CRP have been found to predict future risk among patients with stable and unstable angina,[90–96] in the chronic phase after myocardial infarction,[67] and among patients undergoing revascularization procedures.[97–99]

With regard to risk prediction among patients with acute coronary syndromes, patients presenting with unstable angina who have elevated blood levels of CRP (>3 mg/L) have been found to have higher rate of death, acute myocardial infarction, and need for revascularization procedures compared with patients with CRP levels <3 mg/L.[100] Patients with acute myocardial infarction show a rise in CRP within 6 hours of symptom onset, suggesting that the rise in CRP may be secondary to an underlying proinflammatory state, rather than due to myocardial necrosis. This concept is supported by the finding that patients with coronary vasospasm have persistently normal CRP levels, despite frequent episodes of ST-segment elevation.[101] Moreover, in both short-term[91] and long term studies,[94] CRP has had prognostic value even among patients with acute coronary syndromes with no discernible evidence of myocardial necrosis as evidenced by normal troponin levels. Further evidence in support of a role for inflammatory processes in unstable coronary syndromes comes from data showing that increased levels of IL-1 receptor antagonist and IL-6 at 48 hours after admission are associated with a complicated hospital course.[76] However, given that patients with acute coronary syndromes have already declared themselves to be at increased risk, CRP testing may have greatest clinical utility in the primary prevention setting, where it may be used to guide targeted preventive interventions.

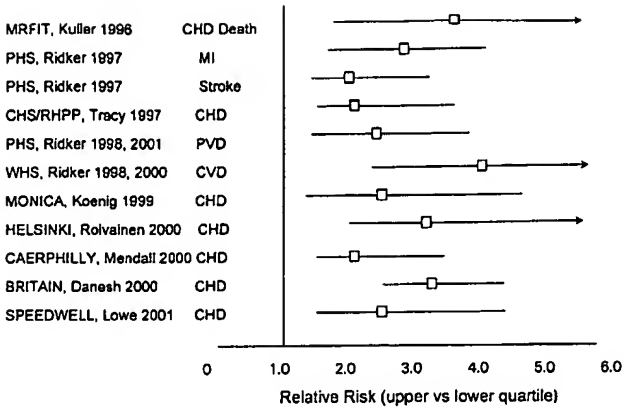

Figure 1. Prospective studies of CRP as a marker of future cardiovascular risk among individuals without known coronary disease. Risk estimates and 95% confidence intervals are calculated as comparison of top versus bottom quartile within each study group. See References 59 through 69. CRP indicates C-reactive protein; CHD, coronary heart disease; MI, myocardial infarction; PVD, peripheral vascular disease; and CVD, cardiovascular disease. Adapted from Ridker.[114]

Data in support of a role for CRP for cardiovascular risk prediction among apparently healthy individuals are robust and remarkably consistent across several European and US cohorts (Figure 1).[79–89] A recent analysis from the WHS sought to compare the risk associated with baseline levels of CRP with other inflammatory and lipid markers of risk. Incident cardiovascular events included death from coronary heart disease, nonfatal myocardial infarction, stroke, and need for coronary revascularization over a mean follow-up of 3 years.[79] Baseline levels of CRP, serum amyloid A (SAA), IL-6, and sICAM-1 were significantly elevated at baseline among the women who subsequently developed cardiovascular events compared with those who did not. Similarly, levels of total cholesterol, LDL cholesterol, and the ratio of total cholesterol to HDL cholesterol (TC:HDL ratio) were significantly higher among patients than control subjects. As shown in Figure 2, of all the inflammatory and lipid markers, CRP was the single most powerful predictor of cardiovascular risk (relative risk for highest compared with lowest quartile=4.4; P<0.001). Multivariate analyses, matched for age and smoking and adjusted for other cardiovascular risk factors, found that only CRP and TC:HDL ratio were independent predictors of future cardiovascular risk. Of both clinical and pathological interest, the addition of CRP, IL-6, SAA, or

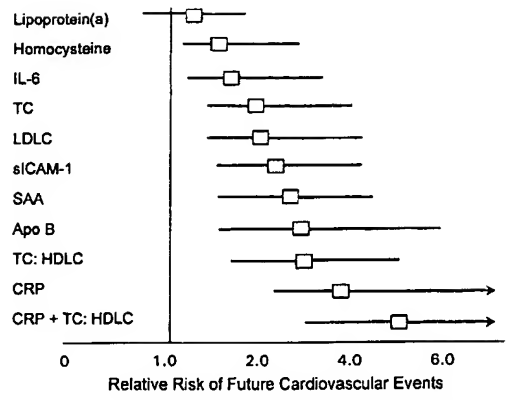

Figure 2. Direct comparison of relative risk of future cardiovascular events associated with levels of lipid and inflammatory risk factors in the Women's Health Study.[79] Relative risks and 95% confidence intervals are shown for women in the top versus the bottom quartile for each factor. IL-6 indicates interleukin-6; TC, total cholesterol; LDLC, low-density lipoprotein cholesterol; sICAM-1, soluble intercellular adhesion molecule-1; SAA, serum amyloid A; Apo B, apolipoprotein B-100; HDLC, high-density lipoprotein cholesterol; and CRP, C-reactive protein. Adapted from Ridker et al.[79]

ICAM-1 testing to lipid testing significantly improved upon risk prediction based on lipid profile alone (Figure 3).

Furthermore, even women with low cholesterol levels were found to be at increased risk if CRP or other inflammatory biomarker levels were high. In a subgroup analysis performed on women with LDL <130 mg/dL, women with increased levels of markers of inflammation were found to be at increased risk for subsequent cardiovascular events, an effect that was strongest for CRP (relative risk for highest compared with lowest quartile=4.1, P=0.002). Similar data have recently been reported for men at risk of developing peripheral arterial disease.[89]

These data suggest that the combination of CRP testing with traditional lipid screening may significantly improve cardiovascular risk prediction, particularly when LDL is low. This might indicate a group among whom aggressive primary prevention therapies should be targeted, such as weight loss, exercise, and smoking cessation. In this regard, CRP levels are known to be higher among patients with several traditional risk factors. Obesity is associated with elevated CRP levels,[102] an observation consistent with the finding that adipocytes secrete IL-6,[103] the main hepatic stimulus for CRP production. Diabetic patients also have elevated CRP levels,[104] which may suggest a role for inflammatory processes in the pathogenesis of diabetes; in this regard, very recent data indicate that IL-6 and CRP levels are elevated at baseline among apparently healthy individuals destined to develop type II diabetes mellitus.[105] Smokers have also been shown to have elevated levels of CRP, IL-6, and sICAM-1.

Accumulating data suggest that the benefits of preventive measures appear to be greatest among individuals with elevated CRP levels. In a large randomized study of aspirin for the primary prevention of cardiovascular events among men, the magnitude of benefit of aspirin in preventing myocardial infarction was directly related to baseline levels of CRP; specifically, the risk reduction for aspirin was 56% (P=0.02) among those with baseline levels of CRP in the highest quartile, while there was a small, nonsignificant reduction (14%, P=0.8) among those with CRP levels in the lowest quartile.[82] This finding raises the possibility that aspirin may prevent ischemic events through clinically important antiinflammatory as well as antiplatelet effects. In a recent study of patients presenting with acute coronary syndromes, CRP was a strong predictor of future risk among those who were not pretreated with aspirin but was not a strong predictor among those who received pretreatment with aspirin.[106] Whether or not aspirin therapy reduces CRP is controversial.[107,108]

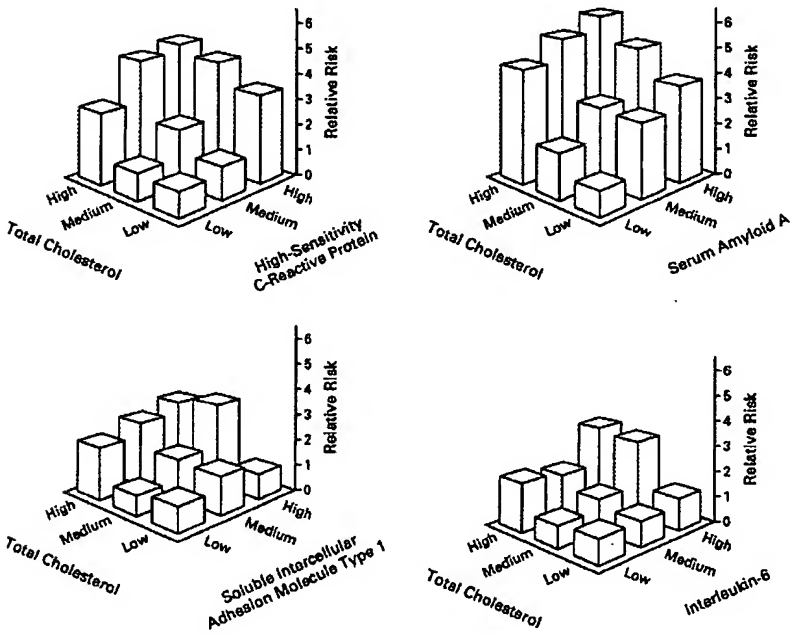

Figure 3. Relative risks of cardiovascular events among apparently healthy women according to baseline levels of total cholesterol and markers of inflammation. Reprinted with permission from Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med.* 2000;342:836–843. Copyright © 2000 Massachusetts Medical Society. All rights reserved.

Clinical Studies of the Antiinflammatory Effects of Statins

Several studies have shown that statin therapy lowers CRP levels independent of its lipid-lowering effects.[62–66] A report from the Cholesterol and Recurrent Events (CARE) study was the first to suggest that the benefit of statins for the prevention of cardiovascular events might be greatest among patients with evidence of persistent inflammation.[67] In the CARE trial, patients with prior history of myocardial infarction were randomized to receive either pravastatin (40 mg) or placebo.[109] Patients with a persistent inflammatory response, as evidenced by elevated levels of both CRP and SAA, were at increased risk for recurrent events. Moreover, the proportion of recurrent events prevented by pravastatin was 54% among those with evidence of persistent inflammation compared with 25% among those without persistent inflammation.[67] These data suggest that statin therapy may be particularly effective among individuals with elevated CRP levels, a finding consistent with the potent antiinflammatory properties of statins.

A recent analysis from the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS),[65] a randomized trial of lovastatin for the primary prevention of cardiovascular events,[110] sought to test this hypothesis in the primary prevention setting. Individuals were divided into 4 groups based on median LDL and CRP levels.[65] Individuals with low LDL (<149 mg/dL) and low CRP (<0.16 mg/dL) levels were at low risk for future cardiovascular events and showed no benefit with lovastatin therapy. Individuals with high LDL (≥149 mg/dL), irrespective of CRP levels, were at a more than 2-fold increased risk for future events and derived substantial benefit from lovastatin therapy (relative risk 0.53; 95% confidence interval 0.37 to 0.77). However, the most interesting data pertained to individuals with low LDL (<149 mg/dL) levels but high CRP (≥0.16 mg/dL) levels. These individuals were at high risk for future events, with a more than 2-fold increased risk compared with those with low LDL and low CRP levels. Furthermore, the benefits of lovastatin therapy were substantial in this group, with a risk reduction (relative risk 0.58; 95% confidence interval 0.34 to 0.98) similar to that seen for individuals with overt hyperlipidemia. These data, although hypothesis generating, suggest that CRP testing may be used to target statin therapy among individuals without overt hyperlipidemia for the primary prevention of cardiovascular disease (Figure 4).

Before CRP screening can be broadly applied in the clinical realm, several limitations of CRP testing require consideration. CRP levels may be transiently elevated for 2 to 3 weeks after a major infection or trauma, and testing should be deferred in this situation. CRP testing may also be of limited value among patients with chronic inflammatory conditions such as rheumatoid arthritis and systemic lupus erythematosus. However, in most studies, less than 2% of all CRP levels have been >1.5 mg/dL, a level that may be associated with an alternative inflammatory condition. For the majority of patients, CRP levels remain relatively stable over a long time period. Indeed, in the CARE trial, the correlation coefficient for two CRP values 5 years apart was 0.6, a value similar or superior to lipid parameters.[62,111,112]

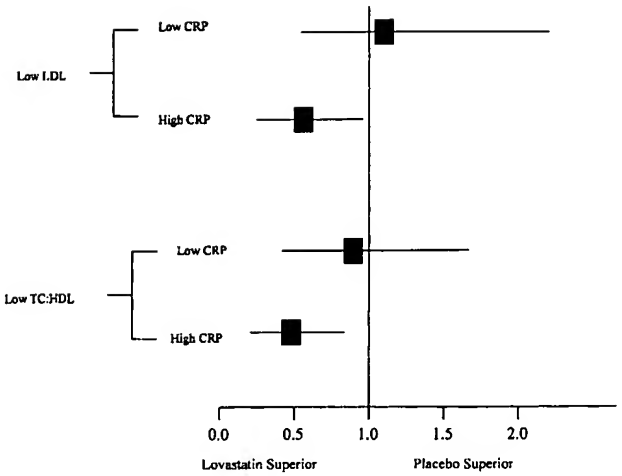

Figure 4. Relative risks (and 95% confidence intervals) associated with lovastatin therapy, according to baseline lipid and CRP levels. LDL indicates low-density lipoprotein cholesterol; TC:HDL, total cholesterol to high-density cholesterol ratio; and CRP, C-reactive protein. Adapted from Ridker et al.[65]

Caution should be used regarding generalization of primary prevention results to secondary prevention populations, given that CRP levels rise substantially during acute ischemia.

Summary

Inflammatory processes play a pivotal role in atherogenesis. Emerging evidence suggests that plasma markers of chronic low-grade vascular wall inflammation may help predict individuals at risk for plaque rupture. Elevated levels of P-selectin, sICAM-1, IL-6, TNF-α, and CRP have been shown to predict future vascular risk in a variety of clinical settings. CRP, a hepatic acute-phase reactant produced in response to IL-6, appears to be the strongest predictor of future cardiovascular risk. Furthermore, the addition of CRP testing to lipid testing may improve upon lipid-based testing alone. Individuals with LDL levels below current treatment guidelines[113] may be at substantially increased risk if CRP levels are elevated.

Recent evidence suggests that statin therapy may be particularly effective among individuals with elevated CRP levels, a finding consistent with the numerous antiinflammatory actions of statins. CRP screening may potentially be used to target statin therapy for the primary prevention of cardiac events among individuals without overt hyperlipidemia.[65]

Acknowledgments

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